

**Micronutrient status in morbidly obese patients prior to Laparoscopic Sleeve
Gastrectomy and micronutrient changes 5 years' post- surgery.**

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Abstract

Background Laparoscopic sleeve gastrectomy (LSG) has become more popular in recent years. The aim of this study was to determine the vitamin and mineral status in patients up to 5 years after LSG, and to explore changes that occurred from preoperatively to 1, 2 and 5 years after surgery.

Methods Data reviewed included age, sex, weight and body mass index (BMI), micronutrient supplements consumed and blood levels of 25 hydroxyvitamin D (25 (OH)D), PTH (parathyroid hormone), ferritin, haemoglobin, folate and vitamin B12, prior to and post-LSG. Data was collated from medical records of morbidly obese patients who had undergone LSG surgery.

Results There were a maximum of 336 patients with preoperative and 1 year after surgery values, n=272 for 2 years and n=116 for 5 years after surgery. At 5 years only 54% (58/107) of patients reported taking daily multivitamin supplements. While most patients had values within the reference range for haemoglobin, vitamin B12, folate and vitamin D five years after LSG, 36 % (34/94) of the patients had serum ferritin below reference value.

Conclusion This study has highlighted a low micronutrient supplementation adherence. Ferritin levels decreased over time even with multivitamin supplementation. To improve micronutrient guidelines prior to and after LSG more research, including controlled supplementation studies are necessary.

Keywords Sleeve gastrectomy - Micronutrient status - Vitamin D - Multivitamins

INTRODUCTION

Bariatric surgery is the only evidence-based long-term treatment option that has shown sustained weight loss and improvement in comorbidities (1). Additional to lowering body weight, bariatric surgery has beneficial effects on type 2 diabetes, blood pressure, hyperlipidimia, stroke and cardiovascular events (2-4). Furthermore, patients after bariatric surgery have a significant reduced mortality compared with controls (5, 6).

The Laparoscopic sleeve gastrectomy (LSG) procedure has become highly popular in recent years and the number of procedures has increased in Europe and in the United States (7).

However, as a relative newcomer there is a paucity of research investigating the incidence of micronutrient status after LSG.

Morbidly obese patients have high occurrence of micronutrient deficiencies compared with healthy weight individuals of same sex and age (7). These deficiencies might be important to detect prior to surgery as these can deteriorate postoperatively and may result into long-term complications (8).

Recent studies conducted up to 2016 have identified a high occurrence of vitamin D (25 (OH) D) deficiencies in patients prior to bariatric surgery. Folate, vitamin B12 and iron levels have also been frequently reported to be low in patients prior to LSG (7-13). Studies investigating anaemia and micronutrient deficiencies in patients after LSG are limited. Vitamin deficiencies are of concern after bariatric surgery, with 50% of reported deficiencies occurring within the first year of surgery (9). The LSG surgery does not seem to affect the digestive system besides from accelerating the gastric emptying and possibly from consequences due to resection of the gastric fundus (14, 15). However, there is still lack of evidence on how LSG affects the gastric emptying and if fast passage of food through the gastrointestinal tract promotes micronutrient deficiencies (14). Removing the gastric fundus during LSG decreases the production of parietal cells that secrete hydrochloric acid which can affect iron absorption and intrinsic factor (IF) that is essential for vitamin B12 uptake (8, 16). Recent studies have

shown that low levels of vitamin B12 and iron are common in patients 1 to 3 years post-LSG (7-9, 11, 17, 18). LSG patients are also at risk of low levels of folate, 25 (OH) D, and ferritin postoperatively (7, 9, 10, 12, 17, 19). Studies investigating long-term micronutrient deficiencies after LSG are few and to our knowledge only a small study (n=30 at 5 years) by Saif *et al.* (2012) is the only publication that has reported micronutrient deficiencies after LSG with a five-year follow-up(19). Hence, there are still uncertainties on which micronutrient deficiencies present in these patients and whether specific micronutrient supplementation is required.

The aim of this study was therefore to determine the vitamin and mineral status in patients up to five years after LSG, and to explore changes that occurred from preoperatively to one, two and five years after surgery. In this study we focus on vitamin B12, folate, 25 (OH)D and iron.

MATERIALS AND METHODS

Data was extracted from the database for bariatric surgery at Førde Central Hospital. This is a database approved by the Norwegian Data Inspectorate and patient inclusion is based on written informed consent allowing data to be used for surveillance and research. Exclusion criteria for surgery included pregnancy and active psychosis. Patients included in this study were operated in the time period between May 2007 and April 2014.

Patients eligibility for LSG was based on international standard criteria for bariatric surgery(20). Systematic follow-up examinations were scheduled at 12, 24 and 60 months after surgery. At every follow-up time, anthropometric measurements and biomaterial sampling were completed. Inclusion criteria for the study was at least one preoperative blood sample and one during the first post-operative year.

Surgical procedure

Prior to surgery, patients were offered a one-day seminar which included information from a clinical nutritionist, nurse, surgeon, psychologist and a previously operated patient.

Estimations of results and information about life changes after surgery as nutrition, smoking and physical activity was provided. A detailed description on how the LSG operation procedure was conducted on the patients at Førde Central Hospita has been published(12).

Post- surgery all patients were advised to consume a low carbohydrate diet, high protein diet, high frequency of water, a multivitamin tablet daily and include physical activity. The first 61 patients were routinely recommended to take Calcigran Forte (NycoMed Pharma TM) post-surgery containing one gram of calcium carbonate and 800IE 25(OH)D daily. At every follow-up all patients were requested to complete a paper form indicating which supplements they were taking. The brand of multivitamin taken was not recorded; however within Norway Nycoplus is the most common brand of vitamin and mineral supplement each tablet includes:

250 µg vitamin A, 10 µg vitamin D, 10 mg vitamin E, 120 µg vitamin K, 75 mg vitamin C, 1,4 mg thiamin, 1,6 mg riboflavin, 19 mg Niacin, 1,5 mg vitamin B6, 200 µg folic acid, 2 µg vitamin B12, 30 µg biotin, 5 mg pantothenic acid, 100 mg magnesium, 15 mg iron, 12 mg zinc, 900 µg copper, 2,3 mg manganese, 60 µg selenium, 35 µg chromium, 150 µg iodine and 45 µg molybdenum.

Clinical and biological assessment

To assess micronutrient status, blood was collected by venepuncture. The biochemical variables were analysed by the Department of Clinical Biochemistry at Førde Central Hospital except for the 25 (OH)D analyses (Hormone Laboratory, Haukeland University Hospital). All analyses were conducted through Architect CI8200, Abbott Diagnostics. 25(OH) D vitamin, ferritin, folate and vitamin B12 were detected in serum samples. Plasma and complete blood count were measured for PTH and haemoglobin. 25(OH) D vitamin, folate and vitamin B12 were assayed by a competitive immunoassay using direct chemiluminescence technology.

Statistical analyses

All analyses were carried out using IBM® Statistical Package for the Social Sciences® (SPSS®) version 21. Values are expressed as mean \pm SD. To examine micronutrient status of the patients at two different time points a paired sample t-test was performed. The repeated measure Anova was conducted to assess the effect of time (pre-operative, 12, 24 and 60 months postoperatively). A p-value of less than 0.05 was used to declare statistical significance.

RESULTS

There were a maximum of 336 morbidly obese patients with preoperative and 1 year after surgery values, n=272 for 2 years and n=116 for 5 years after surgery. For the 336 morbidly obese patients studied (239 women and 97 men) the mean weight prior to LSG was $132.5 \pm$

23.9 kg (standard deviation (SD)), mean BMI $45.3 \pm 6.2 \text{ kgm}^{-2}$, and mean age 41.0 ± 11.7 years (Table 1.). Mean (SD) change in BMI at 12 months was $15.2 \pm 4.7 \text{ kgm}^{-2}$ (n=322), 24 months $15.3 \pm 5.6 \text{ kg/m}^2$ (n=267) and at 60 months mean change in BMI of $13.2 \pm 6.0 \text{ kgm}^{-2}$ (n=107). We have follow-up data for weight in combination with an overview of micronutrient status from 322 patients at 12 months (96%), 267 patients at 24 months (98%) and 107 patients at 60 months (92%). The lower number of patients at 2 and 5 years reflects the total number who had their procedures at least 2 or 5 years before data collection.

Very few patients were taking micronutrient supplements prior to surgery (Table 2.) After surgery the majority of patients were taking multivitamin supplements, dropping to 54% at 60 months. Vitamin B12 supplementation was taken by 20-30% of patients at 12, 24 and 60 months post-surgery.

Micronutrient values prior to laparoscopic sleeve gastrectomy

Prior to LSG 20.4% of patients had serum 25 (OH) D values below the reference range, 6.4% had serum vitamin B12 values below the reference range and 8.8% had serum folate values below the reference value (Table 3). Preoperatively just 8 patients (2.4 %) of n=328 were anaemic, based on haemoglobin less than 11.5g/dl, and all were females. PTH values above reference was not found in any of the patients prior to surgery, nor post-surgery.

Changes over time

Ferritin serum levels were significantly lower at 12, 24 and 60 months compared with preoperative values (Table 4.) Those patients taking a multivitamin supplement at 60 months did have significantly higher ferritin levels compared with those not taking any micronutrient supplement (Table 6. $p=0.003$), patients taking iron supplements were excluded from the

analysis. Haemoglobin levels remain stable over time although levels were slightly lower at 12 and 24 months compared with preoperative values (Table 4).

Serum 25 (OH)D values at 12, 24 and 60 months were significantly greater compared with values prior to surgery (Table 4) and fewer patients had 25 (OH)D serum values below the reference range (Table 3). Those patients taking a multivitamin supplement at 12 months had significantly higher serum 25(OH) D values at 12 months postoperatively compared with those not taking a multivitamin supplement ($p=0.032$), though no differences at 60 months (Table 6). ALP and PTH were stable over time (Table 4).

Serum folate values were also significantly greater at 12, 24 and 60 months compared with values prior to surgery (Table 4). Those patients taking a multivitamin supplement at 12 months had significantly higher serum folate values at 12 months postoperatively compared with those not taking a multivitamin supplement ($p=0.001$) (Table 6).

A larger proportion of patients had serum vitamin B12 values below the reference range at 12 months (19 %) and 24 months (12.8%) compared with the 6.4% prior to surgery, though only 3.8% were below the reference range at 60 months. The mean serum vitamin B12 value significantly decreased at 12months (Table 4).

Discussion

LSG patients are potentially at risk of micronutrient deficiencies due to their substantially reduced dietary intake, decreased hydrochloric acid and intrinsic factor and possibly poor food choices. Therefore, it has been recommended for LSG patients to take daily micronutrient supplements to prevent any deficiencies (16). In the current study 75 and 70% of patients were taking multivitamin supplements 12 and 24 months' post LSG respectively, this dropped to 54% at 5 years.

Mean ferritin levels decreased over time, and prevalence of those below the reference ranges increased over time; similar to previous findings (8, 9, 12, 17). The prevalence of low haemoglobin level 5 years after LSG was 5.2%. These findings are not supported by Saif *et al.* (2012) who reported 28.6% of patients (n=30) having a low haemoglobin level 5 years after LSG surgery(19). In the current study patients with a low ferritin or haemoglobin level after LSG may be explained by malabsorption and a reduction in hydrochloric acid that can potentially affect iron absorption (21, 22) or a restricted dietary iron intake. Aarts *et al.* (2011) found that a quarter of their patients had anemia, over 40 % had iron deficiency despite taking a multivitamin supplement(17). Serum ferritin is an acute phase reactant to inflammatory processes in obese individuals and in this way elevates the level of ferritin (23, 24). In this current study, seen in parallel with postoperative weight-loss, tissue inflammation may have reduced in patients and thereby also the ferritin levels at 1, 2 and 5 years post-surgery.

Mean haemoglobin levels at 5 years were similar to preoperative values, similar to a large study by Aaseth *et al.* (2015) whereby there was no significant change in haemoglobin levels 5 years after Roux-en-Y surgery compared to preoperative values (n=443)(25). 23.7% of the patients were taking iron supplementation 5 years' post-surgery, where as in the current study only 12% were taking iron supplements. Serum ferritin values decreased even in those

patients taking multivitamin supplements, thus clinicians need to consider the value in screening patients to determine who may require iron supplementation.

Intrinsic factor is necessary to release vitamin B12 contained in food; therefore, vitamin B12 deficiencies could be expected with LSG. Deficiencies in vitamin B12 and iron have been noted at 1 year and 3 years post-LSG (7-9, 18). In our study at 12 and 24 months post-LSG a high proportion of patients (19% and 12.8%) had serum levels below the reference range; despite over 70% of patients taking one multivitamin tablet daily.

Hakeam *et al.* (2009) reported the mean vitamin B12 concentration decreased significantly 12 months post LSG with 26% of patients vitamin B12 deficient one year after LSG surgery(9). In addition, these patients were given five times the required daily allowance of oral vitamin B12 and this failed to prevent the postoperative deficiencies. This may indicate that B12 deficiencies could be attributed to fundus resection after LSG, which can cause a reduction in IF and lead to B12 malabsorption. Gehrer *et al.* (2010) detected high B12 deficiencies in patients one year after LSG despite 10µg cyanocobalamin preoperative(10). However, the supplement with intramuscular cyanocobalamin corrected the deficiencies in 91% of the patients. This may indicate that early post-LSG, patients may be recommended to take B12 supplementation in addition to intramuscular, to be effective. Saif *et al.* (2012) did not detect B12 deficiencies at 5 years (n=30), this is confirmed in our study whereby only 3.8 % had serum B12 values below the reference range (n=4/92).

Only 12.3% of patients had folate values below the reference range 12 months after LSG, lower than the 12.5-28.6% reported previously among other LSG studies (7, 10, 17). Folate deficiency after LSG is not primarily caused by the surgery and the primary cause of this deficiency is more assigned to food choices and that folate stores decrease quickly when intake is insufficient (7, 17).

Serum vitamin D increased over time compared to levels prior to LSG surgery. This finding is supported by previous studies(7, 8, 11, 12, 19). This may be explained by several factors. Sixty-one patients were advised to take 800IE vitamin D3 daily postoperatively and every patient were advised to take one multivitamin tablet daily. In addition, Blum *et al.* (2008) showed a positive correlation between serum 25 (OH)D and adipose tissue in obese individuals indicating adipose tissue is a strong 25 (OH)D reservoir and thus storage release of 25 (OH)D due to significant weight loss 12 months post-operatively may explain the increase in mean serum 25 (OH)D values (26). 25 (OH)D values below the reference range improved postoperatively compared to preoperative values and the mean serum 25 (OH)D increased significantly at every time-point postoperatively.

Previous studies have indicated that although patients after LSG are vitamin D deficient normal calcium status as evaluated by PTH and ALP levels are observed (7, 10, 12, 15, 17, 19). The results from this study and supporting evidence from previous studies may indicate that calcium supplementation is not necessary as a routine in the long term, and might only be supplemented if deficiencies are detected (7, 8, 10, 12, 15, 17).

Limitations

This study has its limitations. It is hard to distinguish if the reduction of serum and plasma values below reference range were based on a lack of supplementation, weight loss or dietary intake. The compliance to multivitamin supplementation were not taken into account. In addition, it is not clear if patients were taking additional iron, vitamin B12 or folate supplementation apart from what was documented. Furthermore, the indication for initiating supplementation is not documented as this was started outside specialized care and we do not know whether this was based on serum values. As this study did not conduct a close monitor of patient's type, quantity and frequency of vitamin and mineral intake it makes is hard to

establish if the multivitamin supplementation were inadequate or if the serum and plasma values below reference range are caused primarily by the LSG.

Conclusion

Most patients had values within the reference ranges for haemoglobin, vitamin B12, folate and vitamin D five years after LSG, while 36 % of the patients had serum ferritin below reference value.

Ferritin levels decreased over time even with multivitamin supplementation. The vitamin B12 level was lowest one year postoperatively while the vitamin D status was considerably improved at one year and remained stable thereafter. To improve micronutrient guidelines prior to and after LSG more research, including controlled supplementation studies are necessary.

Statements Regarding Ethics and Consent.

Data was extracted from the database for bariatric surgery at Førde Central Hospital. This is a database approved by the Norwegian Data Inspectorate and patient inclusion is based on written informed consent allowing data to be used for surveillance. All procedures performed in this study were in accordance with the procedures of the University of Roehampton Ethics committee and with the 1964 Declaration of Helsinki and its later amendments.

Conflict of Interest Statement.

All authors have no conflicts of interest to disclose.

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Table 1. Sex, weight, body mass index and age of patients

Variables	Preoperative		12m post-op		24m post-op		60m post-op	
	n/	mean ±SD	n/	mean ±SD	n/	mean ±SD	n/	mean ±SD
Sex (women/men)	239/97		229/92		188/79		80/27	
Weight (kg))	123.5	23.9	87.5	17.4	88.2	18.3	95.1	16.7
BMI (kgm ⁻²)	45.3	6.2	29.9	5.0	30.1	5.4	32.9	5.5
Age (years)	41	11.7	41	11.7	41	11.5	41	11.0

±SD=standard deviation; BMI=body mass index; m post-op. =months post-operative.

Table 2. Percentage of patients reported to be consuming micronutrient supplements up to 60 months after laparoscopic sleeve gastrectomy

Supplements	Preoperative n 336	12m post-op n 322	24m post-op n 267	60m post-op n 107
Multivitamin	10.1	75.8	69.7	54.2
Vitamin B12	3.6	21.4	23.6	29.9
Folic Acid	1.2	3.4	7.9	12.1
Calcium	0.9	12.7	19.5	26.2
Vitamin D	1.2	3.7	3.0	3.7
Iron	1.2	3.4	7.9	12.1

m post-op. =months post-operative.

Table 3. Proportion of patients (%) with biochemical variables below or above reference values prior to and 12, 24 and 60 months after laparoscopic sleeve gastrectomy

Variables with reference range		preoperative	12m post-op	24m post-op	60m post-op
	%	n 270	n 288	n 219	n 90
25 (OH)D vitamin 30-150 nmol/L	deficient	20.4	4.9	8.2	6.7
	normal	78.9	94.8	91.8	93.3
	excess	0.7	0.3	-	-
Ferritin 15-120 ug/L	deficient	n 334 3.3*	n 301 11.6*	n 230 20*	n 94 36.2*
	normal	60.5	55.8	57	57.4
	excess	36.2	32.6	23	6.4
Haemoglobin 11.5-16.0 g/dl	deficient	n 336 2.4	n 308 3.6	n 242 3.7	n 97 5.2
	normal	93.8	92.2	91.7	92.8
	excess	3.9	4.2	4.5	2.1
Folate >6 nmol/L	deficient	n 328 8.8	n 308 12.3	n 236 7.6	n 94 10.6
	normal	91.2	87.7	92.2	89.4
	excess	-	-	-	-
Vitamin B12 175-700 pmol/L	deficient	n 329 6.4	n 306 19.0	n 234 12.8	n 92 3.8
	normal	91.8	78.4	83.8	91.3
	excess	1.8	2.6	3.4	5.4

Results are expressed as percent %; m post-op. =months post-operative. * The percentage of females within the group preoperatively (100%), 12m (97%), 24m (98%) and 60m (91%). 25(OH)D = 25 hydroxyvitamin D. Normal, deficient and excess levels are values based on analyses by the department of Clinical Biochemistry at Førde Central Hospital (except for 25 (OH)D) Hormone laboratory, Haukeland University Hospital. m post-op. =months post-operative.

Table 4. Micronutrient values comparing prior to surgery with 12, 24 and 60 months after laparoscopic sleeve gastrectomy

	n	preoperative	12m post-op	p value	n	preoperative	24m post-op	p value	n	preoperative	60m post-op	p value
25 (OH)D vitamin	225	49.2±25.4	63.2±23.2	<0.001	164	47±28	59±21	0.002	85	43±21	63±24	<0.001
Ferritin	299	122±110	108±106	<0.001	229	116±107	88±96	<0.001	93	108±85	44.6±57.3	<0.001
Female	211	88±76	79±82	0.06	161	80±78	60±71	<0.001	73	86±61	32±33	<0.001
Male	88	205±133	176±126	0.007	68	200±120	154±113	0.001	20	189±108	90±94	0.001
Haemoglobin	308	13.95±1.2	13.62±1.3	<0.001	242	13.9±1.2	13.6±1.3	<0.001	97	13.5±1.2	13.6±1.5	0.691
Folate	300	14±7.8	16.5±10.3	<0.001	228	14.3±8	18.2±11	<0.001	90	12.8±6.7	20.1±35	0.029
Vitamin B12	304	321.6±144	293.9±192	0.021	234	317.7±143.8	326.1±223.8	0.566	90	307±168	541±1453	0.129
Calcium	310	2.4±0.1	2.4±0.1	0.169	240	2.3±0.1	2.3±0.1	0.127	97	2.3±0.1	2.4±0.2	<0.001
ALP	308	76.4±21.9	72.0±21.2	<0.001					94	75.5±20.2	69.4±22.0	<0.001
PTH	281	7.7±3.4	6.8±2.9	<0.001	211	7.9±4.6	7.2±3.4	0.070	79	7±3.1	8.8±5.2	0.002

Values expressed as mean ± standard deviation. Paired t test used to compare pre and post values.
ALP=alkaline phosphatase; PTH=Parathyroid hormone

Table 5. Micronutrient values prior to and, 12, 24 and 60 months after laparoscopic sleeve gastrectomy

Variables with reference ranges	n	preoperative	12m post-op	24m post-op	60m post-op
25 (OH)D vitamin 30-150 (nmol/L)	59	43.4±21.7 ^{a,b}	60.2±25.2	58.7±20.1	62.8±23
Parathyroid hormone 0.7-7.5 (pmol/L)	58	7.2±3.3c	7.2±2.6c	7.6±2.8	8.6±3.7
Ferritin 15-120 (ug/L)	55	106±90.7 ^b	97.8±77.2 ^b	88.4±99.9 ^b	45.2±67.2
Multivit at m60*	28	127±102	115±67	104±107	53±52
No multivit*	20	101±68	97±58	75±52	26±20
Haemoglobin 11.5-16.0 (g/dl)	71	13.6±1.2	13.3±1.1	13.5±1.1	13.7±1.2
Folate >6 (nmol/L)	67	12.6±6.0	17.3±10.6	20.2±11.9	18.4±11.5
Vitamin B12 175-700 (pmol/L)	72	303±174	276±210	365±291	404±251
Multivit at m60*	18	322±99	249±88	323±152	342±99
No multivit*	15	354±170	250±130	304±147	374±243

Results are expressed as mean ± SD and derived from repeated measures ANOVA. ^a significant difference with 24months, ^b significant difference to 60 months.* Excludes patients taking iron supplements at any time point; there was a significant difference over time, * Excludes patients taking vitamin B12 supplements at any time point; there was a significant difference over time. Multivit.= multivitamin and mineral supplement; m post-op. =months post-operative.

Table 6. Micronutrient values prior to, and at 12 and 60 months after laproscopic sleeve gastrectomy in those taking micronutrient supplements and those not.

Vitamin supplement		preoperative		12m post-op		60m post-op	
		N	mean±SD	N	mean±SD	N	mean±SD
25 (OH)D vitamin	Multivitamin	28	56±16	221	63±22*	51	65±27
	Vitamin D	3	106±19	10	69±26	4	40±20
	no supplement	242	47±25	52	55±26*	39	59±19
Ferritin	Multivitamin	33	101±76	233	114±113	51	60±71*
	Ferritin	3	31±21	10	14±7	12	36±32
	no supplement	301	121±110	53	87±76	43	27±23*
Haemoglobin	Multivitamin	34	14±1	238	14±2	52	14±1
	Iron	4	13±2	10	13±1	12	13±2
	no supplement	302	14±1	54	13±1	45	13±2
Folate	Multivitamin	32	21±11*	238	18±10*	51	19±11
	Folate	3	32±7	15	24±15	4	36±5
	no supplement	296	13±7*	54	13±10*	44	23±49
Vitamin B 12	Multivitamin	32	338±164	240	291±186	52	377±251
	Vitamin B12	11	291±100	67	377±295	27	495±341
	no supplement	298	321±141	54	309±218	43	715±2084
ALP	Multivitamin	33	77±23	246	72.5 ± 22.1	52	70.5 ± 24.4
	calcium	3	70.0 ± 8.9	41	70.0 ± 15.5	26	71.1 ± 23.3
	no supplements	301	76±21	54	69.1 ± 17.1	43	67.5 ± 18.8
PTH	Multivitamin	29	6.9 ± 2.2	236	6.6 ± 2.7	52	9.5 ± 6.1
	calcium	2	5.9 ± 0.9	38	7.3 ± 3.5	27	9.5 ± 4.9
	no supplements	280	8.1 ± 4.5	52	7.3 ± 3.2	41	7.6 ± 3.7

Biochemical variables are expressed as mean and ± standard deviation * significant difference between those taking a multivitamin supplement and those not (t test; P<0.05). Individual nutrient supplements were not included in statistical analysis due to small numbers.